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TITLE: Evaluation of an Acute RNAi-Mediated Therapeutic for Visual Dysfunction
Associated with Traumatic Brain Injury

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INTRODUCTION:

Traumatic brain injury (TBI) is the leading cause of death in children and young adults globally. Malignant cerebral edema plays a major role in the pathophysiology which evolves after severe TBI. Added to this is the significant morbidity and mortality from cerebral edema associated with acute stroke, hypoxic ischemic coma, neurological cancers and brain infection. Therapeutic strategies to prevent cerebral edema are limited and if brain swelling persists beyond 24 h, the risks of permanent brain damage or mortality are greatly exacerbated. Currently, hyperosmolar therapy for acute brain swelling involves the administration of the osmotic diuretic mannitol or hypertonic saline which theoretically will reduce cerebral edema by drawing water across areas of intact blood-brain barrier (BBB) into the vasculature, however as well as being short-lived, mannitol infusion may promote complications associated with severe intravascular volume depletion, hypotension and hyperkalemia.

We have discovered a process whereby levels of the tight junction protein claudin-5 at the BBB can be suppressed in the microvasculature of the brain using RNAi. Temporary and size-selective modulation of the BBB allows enhanced movement of water from the brain to the blood and significantly impacts on the formation of cerebral edema. Moreover, we also show cognitive improvement in mice with focal cerebral edema following administration in these animals of siRNA directed against claudin-5. These observations may have profound consequences for early intervention in cases of TBI, or indeed any neurological condition where cerebral edema is the hallmark pathology. Indeed, we are now set to begin the clinical research arm to this study whereby cerebral edema development will be monitored using MRI in a cohort of patient who have suffered out of hospital cardiac arrest.

BODY:

Task 3. Analysis of systemic biomarkers of TBI in mice and assessment of cell death at the site of injury (July 2010-July 2013)

All blood samples have been gathered for the analysis of S100B and neuron specific enolase (NSE) and this assessment will be completed in approximately 1 month. Cell death at the site of injury was assessed using TUNEL labelling of brain cryosections and this data was published in our Nature Communications paper, details of which are outlined below.

Task 4. Clinical assessment of individuals with homogeneous cerebral edema formation (July 2010-July 2013)

Since the granting of ethical approval for this trial in October 2012 12 patients have been recruited to this trial. Of these 2 MRI scans were performed in 8 patients. In addition a number of other patients were screened for inclusion in the trial but were excluded for the following reasons:

- MRI incompatible duodenal ulcer clips
- Sternal wound dehiscence requiring vac dressing
- Chylothorax requiring chest drain

- Pneumothorax requiring chest drain
- Cardiac pauses requiring pacemaker
- Refusal of consent - Claustrophobia
- Spinal Cord Injury
- Unstable due to tracheal stenosis and lobar collapse
- 2 patients had treatment withdrawn due to poor prognosis and subsequently passed away
- 1 patient became uncomfortable in the MRI machine and asked for the scan to be stopped.
- Heart Block necessitating pacemaker
- Unstable from a respiratory point of view due to previous Left lower lobectomy, right upper lobectomy and extensive pneumonia.
- Multiple previous strokes
- Suspected swine flu necessitating infection control precautions
- Multiple metal fragments

The analysis of the patient information collected so far has allowed us to perform Voxel Based Morphometry on the MRI data. This was performed between the patients first and second scan but also between the patient's first scan and age and gender matched controls. This was looking for differences in the volume of grey and white matter between scans, however no significant differences were detected.

Additional analysis of the MRI data demonstrated significant differences in the Default Mode Network of patients between first and second scan, which will be further evaluated between patients with a poor neurological outcome and those who survived.

Additional analysis of neurophysiological, biochemical and other imaging sequences will be incorporated shortly to identify other associations in data

KEY RESEARCH ACCOMPLISHMENTS:

Recruitment of 12 patients into trial.

Publication of research findings: Campbell, M, Hanrahan, F, Gobbo, OL, Kelly, ME, Kiang, AS, Humphries, MM, Nguyen, ATH, Ozaki, E, Keaney, J, Blau, CW, Kerskens, CM, Cahalan, SD, Callanan, JJ, Wallace, W, Grant, GA, Doherty, CP and Humphries, P. Targeted suppression of claudin-5 decreases cerebral edema and improves cognitive outcome following traumatic brain injury. *Nature Communications*, (2012), May 22;3:849.

REPORTABLE OUTCOMES:

- Claudin-5 siRNA/in vivo jet PEI was designated an orphan medicinal product by the European Medicines Agency (EMA) for its use as a platform to enhance drug delivery to glioblastoma multiforme (GBM) brain tumours.
- A full safety/toxicology profile of claudin-5 siRNA/in vivo jetPEI has now been obtained in a non-human primate model.

- Applications will shortly commence for a clinical trial evaluating the safety/tolerability of systemic claudin-5 siRNA/in vivo jetPEI in healthy human volunteers.

CONCLUSION:

Due to delays in obtaining ethical approval a no cost extension was requested and approved to facilitate additional recruitment. This will continue to April 26th 2014 (research ends March 26th 2014).

We have proven that systemic injection of siRNA directed against claudin-5 can relieve cerebral edema associated with a murine model of TBI. This finding has now provided a platform to extend these basic research findings to a clinical setting.

"So what section"

Our findings from the research support of TATRC have led to the development of a novel therapeutic strategy for the treatment of cerebral edema. We are now in the process of extending this technology to a range of neurological conditions that have cerebral edema as a hallmark feature. This new medicinal product, claudin-5 siRNA, should lead to a better prognosis for individuals suffering cerebral edema and we hope to initiate clinical trials for its use in the short term.

REFERENCES:

Campbell, M, Hanrahan, F, Gobbo, OL, Kelly, ME, Kiang, AS, Humphries, MM, Nguyen, ATH, Ozaki, E, Keaney, J, Blau, CW, Kerskens, CM, Cahalan, SD, Callanan, JJ, Wallace, W, Grant, GA, Doherty, CP and Humphries, P. Targeted suppression of claudin-5 decreases cerebral edema and improves cognitive outcome following traumatic brain injury. *Nature Communications*, (2012), May 22;3:849.

APPENDICES: N/A

SUPPORTING DATA: N/A